Synthesis of Undecagold Cluster Molecules as Biochemical Labeling Reagents. 3. Dimeric Cluster with a Single Reactive Amino Group†

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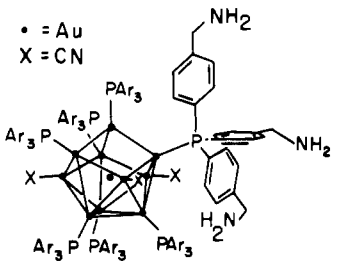
ABSTRACT: The synthesis and characterization of a dimeric derivative of the undecagold cluster complex $Au_{11}(CN)_3[P(C_6H_4CH_2NH_2)_3]_7$, **1**, are described. The dimer, **2**, consists of cross-linked molecules of **1** in which 41 of the 42 amino groups are acylated, leaving a single free amino group per dimer. This amino group is reactive and can be used to prepare derivatives of **2** for use in labeling biological macromolecules in preparation for electron microscopic analysis. Limited acylation of **1** by 1.3 equiv of citraconic anhydride in aqueous solution, followed by extensive acylation with acetic anhydride at pH 7–7.5, leads to a mixture of monomeric and dimeric products. Hydrolytic removal of *N*-citraconyl groups at pH 3.2 unmasks a few amino groups. Cation-exchange chromatography at pH 7 separates the products into three main groups, species containing one, two, or three free amino groups, in overall yields of 33%, 28%, and 15%, respectively. The monoamino species consist mainly of **2** and a monomeric product, which are separated by gel exclusion chromatography. **2** migrates as a dimer upon polyacrylamide gel electrophoresis in sodium dodecyl sulfate, and it contains a single free amino group, detectable by its ion-exchange behavior as a monocation at pH 7 and by its reactivity with [^{14}C]phthalic anhydride.

The preceding papers (Reardon & Frey, 1984; Yang et al., 1984) describe syntheses of undecagold cluster complexes that contain a single reactive carboxyl group, an activated carboxyl group, a single reactive amino group, or a single alkylating functional group such as the bromoacetyl or maleimide groups. These reagents, synthesized from the parent compound $Au_{11}(CN)_3[P(C_6H_4CH_2NH_2)_3]_7$, **1**,[1] can be used for covalently



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labeling proteins in complex biochemical structures in preparation for electron microscopy.

The parent compound and its derivatives can be visualized by scanning transmission electron microscopy (Wall et al., 1982; Safer et al., 1982). A complex of larger size with greater electron scattering potential could be useful for specialized applications or possibly for low-dose conventional transmission electron microscopy. A specialized application might involve specific labeling of one component in a complex structure with **1** and another component with a larger cluster. Scanning transmission electron microscopy could distinguish the two labels and thereby give information about the structural relationships between the labeled sites. A larger structure might also be useful in low-dose conventional transmission electron microscopy.

In this paper we describe the synthesis of a dimeric undecagold cluster, **2**, a complex that consists of two molecules of **1** cross-linked through two amino groups by a methylmalyl moiety and with 39 of the remaining amino groups acetylated, leaving a single free amino group. This complex contains 22 atoms of gold and two ligand spheres and has a molecular weight of about 11 000. The free amino group provides a convenient site to which biochemically specific or chemically selective protein-modifying groups may be attached.

Experimental Procedures

Materials. **1** was synthesized by the procedure of Bartlett et al. (1978). Icosa(*N*-acetyl)-**1** was synthesized by Yang et al. (1984). Citraconic anhydride was purchased from Aldrich Chemical Co. and acetic anhydride from Fisher Chemical Co. [*carboxy*-^{14}C]phthalic anhydride was purchased from Amersham, diluted with carrier, and resublimed. SP-Sephadex C-25, QAE-Sephadex Q-25, and Sephadex G-10 were purchased from Sigma Chemical Co. and Bio-Gel P-6 from Bio-Rad Laboratories.

Analytical Methods. Monomeric and multimeric species of **1** were analytically separated by polyacrylamide gel electrophoresis in the presence of SDS. The procedure followed was essentially that of Weber & Osborne (1969) except that 0.05 M sodium glycinate at pH 10.6 was used as the buffer for casting the gels and as the electrolyte for electrophoresis. This pH, which was higher than those employed for conventional separations of proteins, was near the minimum pH at which henicosa(*N*,*N*-dimethyl)-**1** could be dissolved in buffers containing SDS. At lower pHs serious problems with aggregation were encountered, presumably due to electrostatic interactions between SDS and **1**, which is polycationic below pH 10. Aggregation would presumably not occur with peracetylated derivatives of **1**, but all electrophoresis runs were nevertheless carried out at pH 10.6, which proved to be sat-

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[1] Abbreviations: **1**, tricyanoheptakis[4,4′,4″-phosphinidynetris(benzenemethanamine)]undecagold; icosa(*N*-acetyl)-**1**, **1** with 20 amino groups acetylated and one free amino group; mono[*N*-(*p*-maleimidobenzoyl)]icosa(*N*-acetyl)-**1**, **1** with 1 amino group *p*-maleimidobenzoylated and 20 amino groups acetylated; nondeca(*N*-acetyl)-**1**, **1** with 19 amino groups acetylated and 2 free amino groups; henicosa(*N*,*N*-dimethyl)-**1**, **1** with all 21 amino groups dimethylated; mono(*N*-succinyl)-**1**, **1** with a single amino group succinylated; **2**, 2 molecules of **1** cross-linked by a *N*,*N*′-methylmalyl moiety with 39 of the remaining 40 amino groups acetylated and a single free amino group; *N*-carboxymethyl-**2**, **2** with the amino group carboxymethylated; *N*-phthalyl-**2**, **2** with the amino group phthalylated; DMF, *N*,*N*-dimethylformamide; SDS, sodium dodecyl sulfate.

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isfactory for peralkylated and peracetylated derivatives of **1**. **1** itself did not migrate as a sharp band under any of our trial conditions. Acrylamide (15%) with bis(acrylamide) was polymerized in this buffer supplemented with 0.1% SDS. After electrophoresis the gels were scanned at 280 nm by using an ISCO Model 1310 photometric gel scanner equipped with an ISCO UA-5 absorbance/fluorescence monitor to detect bands containing gold complexes. Concentrations of solutions containing **1** and its derivatives were calculated from measurements of A_{415} assuming the extinction coefficient 2.95×10^4 M^{-1} cm^{-1} at this wavelength (Reardon & Frey, 1984). Radiochemical assays on aqueous samples were performed by combining 1.0 mL of sample with 15 mL of Aquasol and counting in a Beckman LS 100C liquid scintillation spectrometer.

Synthesis of **2**. A solution consisting of 10.2 μmol of **1** dissolved in 1 mL of water and adjusted to pH 7.4 was placed inside a 50-mL pear-shaped flask, together with a small magnetic stirring bar, and purged with a slow stream of N_2 for 30 min. Citraconic anhydride (13.2 μmol) dissolved in dry acetonitrile was added at 25 °C; the solution was mixed quickly and the pH monitored and maintained between 7.0 and 7.5 by microliter additions of 1 M NaOH as required. The pH became stable within a few minutes. After the solution was diluted with water to 20 mL, acetic anhydride (1 mmol, 100 μL) was added to the solution, which was continuously stirred and maintained between pH 7.0 and pH 7.5 by additions of 4 M NaOH until the pH became stable. The addition of acetic anhydride was repeated four additional times. The reaction mixture was adjusted to pH 3.5 by addition of acetic acid and maintained at 25 °C for 3 h. The pH was then adjusted to 7.0 by addition of 4 M NaOH. The mixture was concentrated to 3–4 mL by rotary evaporation in vacuo using a Büchi apparatus, with a bath temperature of 30 °C. $NaBH_4$ (50 μmol) dissolved in 1 mL of water was added to the concentrated solution, which was then desalted by gel filtration through a 4 × 25 cm column of Sephadex G-10 equilibrated and eluted with water. Elution of gold complexes was monitored visually and by A_{415} measurements. The orange-red band was collected and the solution purged with stream of N_2 for 30 min. It was then passed through a 1.5 × 14 cm column of SP-Sephadex C-25 in the Na^+ form, which absorbed the cationic gold complexes as a sharp band at the top. The column was eluted at pH 7 with a linear gradient of NaCl increasing from 0.0 to 0.4 M and formed from 400 mL of each component. Fractions 4.1 mL in volume were collected and A_{415} measurements made on selected fractions. The elution profile is shown in Figure 1.

Fractions 13–27 containing the gold complexes in the first band were pooled, concentrated by rotary evaporation in vacuo, and desalted by gel filtration through a 1.5 × 29 cm column of Sephadex G-10 equilibrated and eluted with water. The desalted material was concentrated by rotary evaporation in vacuo to less than 1 mL. This solution, containing 3.4 μmol of gold complex, was subjected to molecular exclusion chromatography through a 1.1 × 30 cm column of Bio-Gel P-6 (100–200 mesh) equilibrated and eluted with water at a flow rate of 0.2 mL min^{-1}. Fractions 0.4 mL in volume were collected and A_{415} measurements made on selected fractions. The elution profile in Figure 2A revealed two minor bands followed in fractions 40–49 by a major band. The latter fractions were pooled (2.8 μmol), concentrated by rotary evaporation in vacuo to less than 1 mL, and further fractionated through a 1.5 × 29 cm column of Bio-Gel P-6 of finer mesh (200–400) equilibrated and eluted with water at a flow

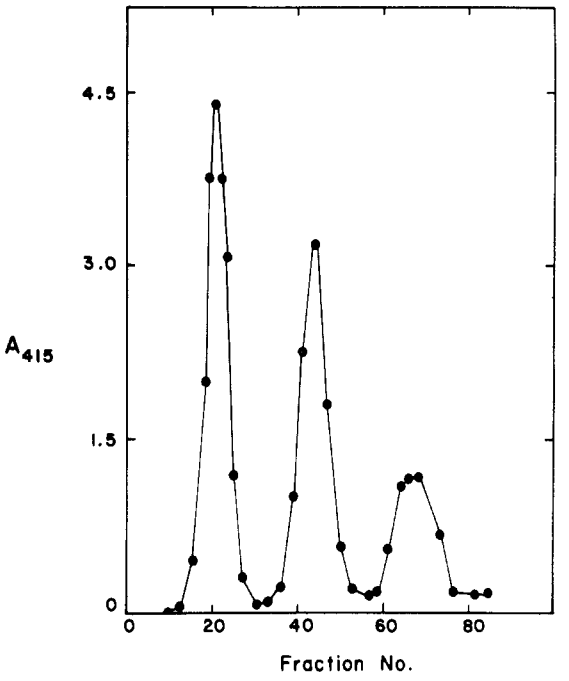


FIGURE 1: Ion-exchange chromatography of the reaction mixture. The reaction mixture for the synthesis of **2** is described under Experimental Procedures. The product mixture was subjected to cation-exchange chromatography through a column of SP-Sephadex C-25 eluted with a gradient of NaCl. The first band emerging from the column contained **2**. Details of the chromatography are given under Experimental Procedures.

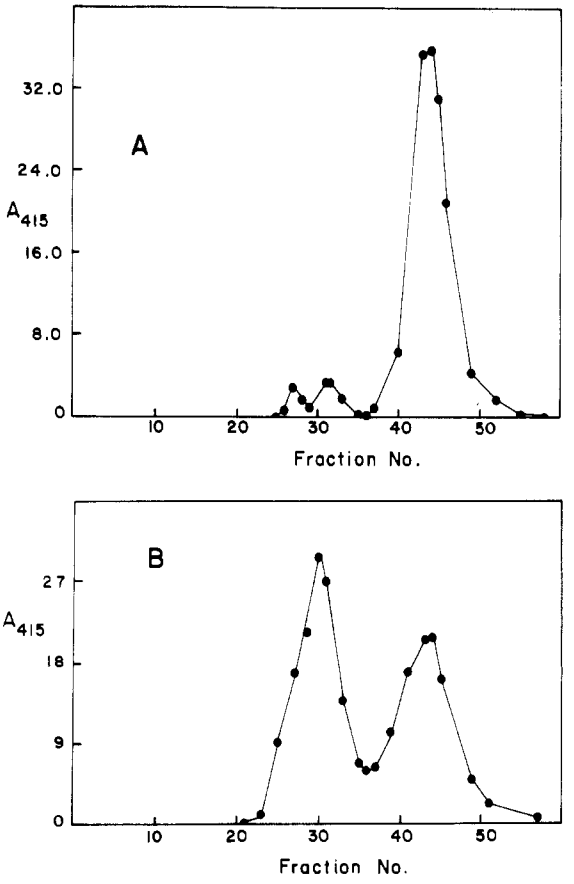


FIGURE 2: Gel exclusion chromatography through Bio-Gel P-6. (Part A) The first peak from Figure 1 was passed through a column of Bio-Gel P-6 (100–200 mesh). The third band emerging from this column contained **2**. (Part B) The third band from part A was rechromatographed through a column of Bio-Gel P-6 (200–400 mesh). The first band contained homogeneous **2**. Chromatographic details are given under Experimental Procedures.

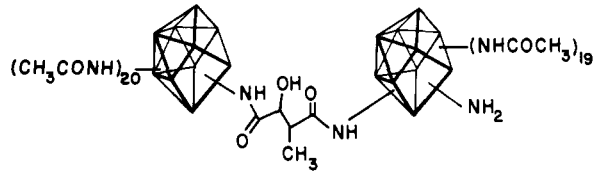
rate of 0.5 mL min^{-1}. Fractions 0.22 mL in volume were collected and A_{415} measurements made on selected fractions. The elution profile in Figure 2B showed two well-resolved bands, one in fractions 23–35 and a second in fractions 37–50. The first band contained **2** in 13% overall yield with respect to that of starting parent compound **1**. The second band contained 1.2 μmol of icosa(*N*-acetyl)-**1**. These products were obtained in sufficiently concentrated form for most purposes and were stored at −70 °C.

Acylation of **2** *by* [^{14}*C*]*Phthalic Anhydride.* A sample of **2** was acylated with an excess of [^{14}C]phthalic anhydride and the *N*-[^{14}C]phthalyl species isolated by anion-exchange chromatography. A 0.05-μmol sample of **2** dissolved in water was purged with N_2 for 30 min. The pH was adjusted to 10 by addition of NaOH and the solution evaporated to dryness. The residue was dissolved in 25 μL of dry DMF, and 0.25 μmol of [^{14}C]phthalic anhydride (1.81×10^6 cpm μmol^{-1}) dissolved in 25 μL of DMF was added. The reaction mixture was stirred for 2 h at 25 °C. After 0.25 μmol of aqueous $NaBH_4$ was added, salts and DMF were removed by gel filtration through a 1.5 × 29 cm column of Sephadex G-10 equilibrated and eluted with water. Fractions containing material absorbing light at 415 nm were pooled and passed through a 0.4 × 2 cm column of QAE-Sephadex Q-25 in the Cl^- form, which absorbed the negatively charged *N*-[^{14}C]phthalyl gold complex. After the column was washed with 1 mL of water, the gold complex was eluted with 0.03 M NaCl at pH 7. By use of the A_{415} measurement and radiochemical assay, the specific radioactivity of the compound was calculated to be 1.83×10^6 cpm μmol^{-1}, assuming the extinction coefficient 5.90×10^4 M^{-1} cm^{-1} for a dimeric derivative of **1**.

Test for Alkylation Reactivity by **2**. A 0.05-μmol sample of **2** in 0.7 mL of water was adjusted to pH 11.3 by addition of NaOH and reduced to dryness by rotary evaporation in vacuo. After the residue was dissolved in 50 μL of DMF, 0.25 μmol of *N*-acetyl[^{14}C]cysteine (8.2×10^4 cpm μmol^{-1}) together with 0.25 μmol of $NaBH_4$ dissolved in 25 μL of water was added. After 1 h at 25 °C, 0.6 mL of water was added and the solution passed through a 1.2 × 32 cm column of Sephadex G-10 equilibrated and eluted with water. Fractions containing **2** were subjected to radiochemical analysis and contained no detectable radioactivity. A portion of the desalted material (0.012 μmol) was adjusted to pH 11 and passed through a small column of QAE-Sephadex Q-25 equilibrated at pH 11. All of the complex passed through the column and was accounted for in the column wash.

Results

Synthesis of **2** *Dimeric Complex.* A structural representation of **2** is given; it is a chromatographically homogeneous,



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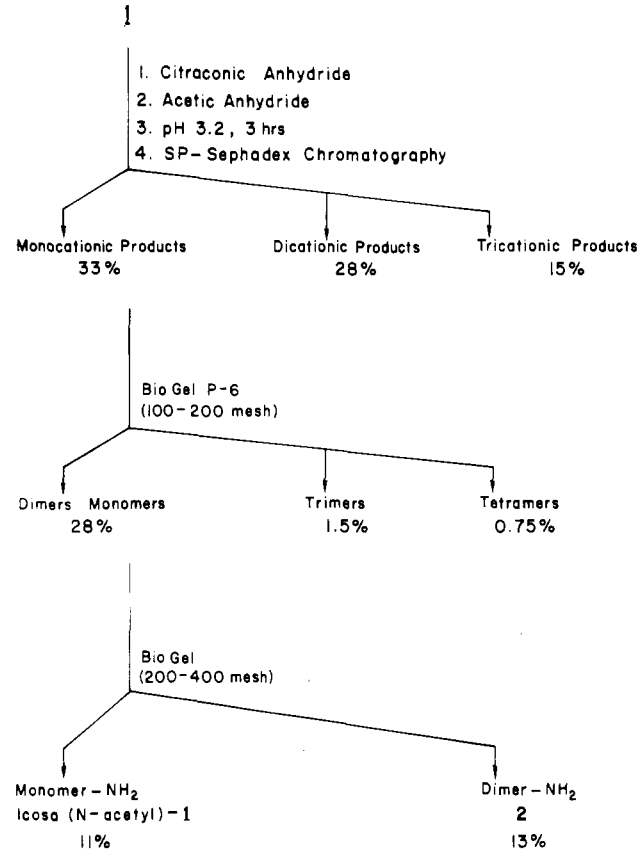
dimeric undecagold cluster with a single primary amino group. **2** is otherwise not chemically or structurally homogeneous for two reasons. First, the amino groups in **1** are not structurally equivalent, so that any one of them can be the unacylated amino group in **2** and any two can be cross-linked. Second, there are probably a few intracluster cross-links as well as the intercluster cross-link shown above.

Scheme I



The flow chart in Scheme I outlines the synthesis and purification of **2**. Acylation of **1** by 1.3 equiv of citraconic anhydride produces a mixture of mono(*N*-citraconyl)-**1**, di-(*N*-citraconyl)-**1**, etc. Treatment of this mixture with acetic anhydride leads to acetylation of amino groups and simultaneously also to the introduction of cross-links involving the *N*-citraconyl groups. Cross-linking is presumably a consequence of the generation of mixed anhydrides arising from reactions of the *N*-citraconyl carboxylate groups with acetic anhydride. The mixed anhydrides acylate amino groups and thereby create cross-links that are in part intermolecular leading to dimeric and higher undecagold species.

The mixed anhydrides can also react with amino groups in a second way, to transfer the acetyl group. This results simply in N-acetylation and regeneration of the *N*-citraconyl group with no cross-linking. Both reactions probably occur, but intermolecular cross-linking leads to dimeric clusters.

The fully acetylated product consists of a mixture of mono-, di-, and tri(*N*-citraconyl) species that is largely monomeric and dimeric with respect to **1**. Hydrolytic removal of *N*-citraconyl groups at pH 3.2 unmasks one, two, or three primary amino groups per dimeric or monomeric entity. Treatment at alkaline pH (10.5) converts the *N,N'*-(1,4-citraconyl) cross-links to *N,N'*-(methyl-1,4-malyl) cross-links by addition of water to the double bond. Cation-exchange chromatography through a column of SP-Sephadex C-25 at pH 7 separates monoamino, diamino, and triamino species as mono-, di-, and trications. This separation, illustrated in Figure 1, shows that 33% of the product consists of monoamino species. Diamino and triamino species are isolated in 28% and 15% yields, respectively. Each band separated in Figure 1 consists of a mixture of monomeric and dimeric entities, with very small amounts of higher oligomers.

Gel exclusion chromatography of the monoamino species, band 1 from Figure 1, through a column of Bio-Gel P-6,

FIGURE 3: SDS–polyacrylamide gel electrophoresis of band 3 from Figure 2A. (Top) The optical scan of the gel loaded with gold complexes from the third band in Figure 2A. (Bottom) The same sample supplemented with icosa(*N*-acetyl)-**1**, showing enhancement of the monomeric component.

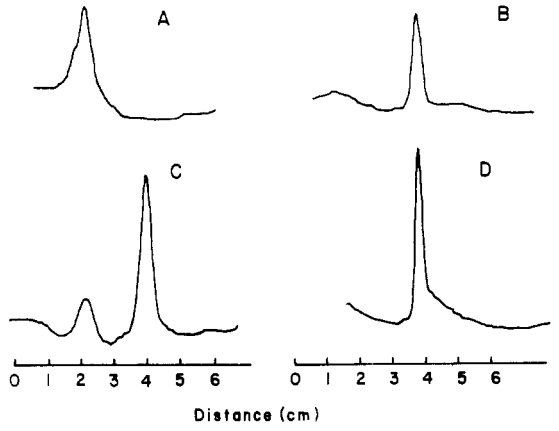


FIGURE 4: SDS–polyacrylamide gel electrophoresis of products from Figure 2B. Shown are optical scans of gels loaded with gold complexes. (Part A) The sample was the first product eluted in Figure 2B. (Part B) The second product eluted from Figure 2B. (Part C) Same as part A but supplemented with icosa(*N*-acetyl)-**1** and showing monomer–dimer separation. (Part D) Same as part B but supplemented with icosa(*N*-acetyl)-**1** and showing enhancement.

100–200 mesh (Figure 2A), separates the gold complexes into three components, two minor ones containing trimeric and tetrameric material and a major band containing a mixture of monomeric and dimeric complexes. This composition is verified by Figure 3, which shows optical scans of gels after SDS–polyacrylamide gel electrophoresis of a sample of the major band and also a second sample supplemented with monomeric icosa(*N*-acetyl)-**1**. Two components are seen, the faster migrating component being enhanced by added, authentic monomer.

The monomeric and dimeric species are separated by rechromatography through a column of Bio-Gel P-6, this time 200–400 mesh. The separation is depicted in Figure 2B. The first product emerging from this column is **2**, the dimeric cluster with a single amino group, and the second is monomeric material. This is confirmed by the optical scans of SDS–polyacrylamide electrophoresis gels in Figure 4. Parts A and B demonstrate the relative mobilities of the two products, showing that the dimer migrates more slowly than the monomer. Part C shows the difference between the mobilities

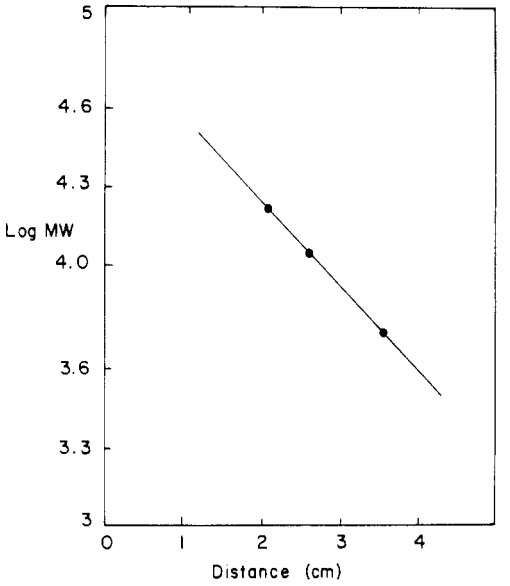


FIGURE 5: Molecular weight calibration of multimeric gold complexes. Samples of icosa(*N*-acetyl)-**1**, **2**, and trimeric **1** from the second band eluted in Figure 2A were mixed and subjected to SDS–polyacrylamide gel electrophoresis. The fastest migrating species was monomer. The log of molecular weight is plotted vs. migrating distance.

of the first product and added, authentic monomeric cluster. Part D demonstrates coelectrophoresis of the second product with authentic monomer.

Purification of **2** from monomers and higher oligomers requires chromatography through both grades of Bio-Gel P-6 in sequential steps. Gel filtration through the 100–200 mesh medium separates trimers and higher oligomers but not monomers and dimers, which are separated by using the finer grade (200–400 mesh). Dimers and trimers are not separated by the finer grade of P-6, so it is necessary to use both grades. The order in which the two columns are used for the separation is immaterial, however, since the reverse order gives equivalent results.

Characterization of **2**. Assignment of dimeric structure to **2** rests upon its migration rate relative to monomer and trimer in polyacrylamide gel electrophoresis experiments, its elution volume from columns of Bio-Gel P-6, and its reactivity with [^{14}C]phthalic anhydride. The separations of monomer, dimer, and trimer by SDS–polyacrylamide gel electrophoresis are quite distinct and consistent, with the relative molecular weights based on elution volumes from columns of Bio-Gel P-6. The molecular weight calibration plot in Figure 5 demonstrates the validity of SDS-polyacrylamide gel electrophoresis for assigning dimeric and trimeric structure to these products.

A partial chemical characterization was fully in agreement with the dimeric structure and verified the presence of a free amino group. Reaction of **2** with excess [^{14}C]phthalic anhydride (1.81×10^6 cpm μmol^{-1}) in DMF produced *N*-[^{14}C]-phthalyl-**2** which was purified by anion-exchange chromatography on a column of QAE-Sephadex. The specific radioactivity of this product was calculated on the basis of A_{415} and radioactivity measurements. The calculation required knowledge of the extinction coefficient of **2** at 415 nm. On the basis that the visible absorption spectrum of **2** is indistinguishable from that of **1**, and this is also the case for all of the other derivatives of **1** that we have described (Reardon and Frey, 1984; Yang et al., 1984), we assumed the extinction coefficient of **2** to be 5.90×10^4 M^{-1} cm^{-1}, twice that of **1**. On the basis of this value, the calculated specific radioactivity of *N*-[^{14}C]phthalyl-**2** was 1.83×10^6 cpm μmol^{-1}. This value

was indistinguishable from the specific radioactivity of [^{14}C]phthalic anhydride, confirming the nature of **2** as a dimer with a single reactive amino group.

The chemical nature of the cross-linkers in **2**, both inter- and intramolecular, is important with reference to the potential applications of derived forms of **2** as protein-modifying agents. The cross-links originate with *N*-citraconyl groups which, as Michael acceptors, are themselves alkylating agents. We contend that the cross-linkers in **2** as we prepare it are hydrated products; that is, the cross-linkers are *N*,*N*′-methylmalyl groups. We base this conclusion on the chemistry of *N*-maleylamines as well as on the fact that **2** exhibits no significant reactivity as an alkylating agent.

In numerous experiments in which we maleylated **1** by reaction with maleic anhydride, we noted that the alkylating properties of *N*-maleyl derivatives of **1** disappeared within a few days in aqueous solutions. Experiments with *N*-maleylbenzylamine confirmed this and also showed that the ultraviolet absorption band associated with the α,β-unsaturated acyl group disappears within minutes at pHs above 10.5.

We also confirmed that **2** itself does not react as an alkylating agent. A sample of **2** was incubated with an excess of *N*-acetyl[U-^{14}C]cysteine under conditions comparable to those under which mono[*N*-(*p*-maleimidobenzoyl]icosa(*N*-acetyl)-**1** reacts (Yang et al., 1984). The reaction mixture was passed through a column of Sephadex G-10 to separate the gold complex from unreacted *N*-acetyl[^{14}C]cysteine. Radiochemical analysis of the complex showed that it contained no detectable radioactivity. **2** could not, therefore, have alkylated *N*-acetyl[^{14}C]cysteine. This was confirmed by passing a portion of the gel-filtered reaction mixture through a QAE-Sephadex column at pH 11. At this pH both *N*-acetyl-[^{14}C]cysteine and gold complex that had alkylated *N*-acetyl[^{14}C]cysteine would be absorbed by the ion exchanger. None of the gold complex was retained by the column, and the column flow through containing the complex was not radioactive.

Dimers of **1** could conceivably also arise by Michael addition of an amino group in a molecule of **1** to the citraconyl group of a citraconylated species of **1**. Our results indicate that this is not a significant cross-linking process under our conditions. Cross-linking by Michael addition would produce species with free carboxyl groups that would be absorbed by QAE-Sephadex at pH 11; however, **2** exhibits no affinity for QAE-Sephadex. Since various derivatives of **1** with free carboxyl groups, including *N*-[^{14}C]phthalyl-**2**, are absorbed by QAE-Sephadex, we conclude that **2** itself contains no carboxyl groups and Michael addition is not involved in the cross-linking process.

Discussion

The simplicity and ease with which **2** can be prepared by the procedures here described may be attributed to two factors, the use of citraconic anhydride as a temporary, easily removable protecting group and the use of acetic anhydride at pH 7 to acetylate remaining amino groups and promote the introduction of cross-links. The ease with which the citraconyl groups are hydrolytically removed, previously noted by Dixon & Perham (1968), facilitates the unmasking of amino groups and the purification of products by cation-exchange chromatography. This aspect of the procedure is similar to what was found with 2,3-dimethylmaleic anhydride (Yang et al., 1984). The *N*-citraconyl groups also participate in cross-linking, as do the *N*-succinyl groups in mono(*N*-succinyl)-**1** (Reardon & Frey, 1984).

The control of pH to below 7.5 during addition of acetic anhydride is probably also an important factor promoting cross-linking and dimer formation. The titration curve for **1** shows that the amino groups exist largely as ammonium ions below pH 7.5 (Reardon & Frey, 1984). In this form they do not react with acetic anhydride, so the acetylation of amino groups is much slower than it would be at a higher pH. The carboxylate anions associated with the *N*-citraconylate groups are quite reactive with acetic anhydride under these conditions, however, and so become activated as mixed anhydrides which eventually participate in the acylation of amino groups, thereby cross-linking them.

Although succinylated and citraconylated derivatives of **1** undergo dimer and oligomer formation during acetylation of the remaining amino groups by reaction with acetic anhydride in neutral solution, 2,3-dimethylmaleylated derivatives remain monomeric under identical conditions (Yang et al., 1984). The reasons for this difference are not known.

We implied in an earlier section that a few intracluster cross-links probably exist in **2**. These would result from reactions of acetic–*N*-citraconyl mixed anhydrides with amino groups in the same cluster. Space filling models show that, owing to steric strain, such linkages are highly improbable or impossible between two amino groups within the same 4,4′,4″-phosphinidynetris(benzenemethanamine) ligand.

Cross-links between amino groups associated with two of the ligands bound to adjacent gold atoms would be unstrained, however; they are, therefore, likely to be present in small amounts. The frequency with which such cross-links appear must be quite small because of the fact that the mole ratio of citraconic anhydride to **1** is only 1.3.

The overall yield of **2**, about 13%, may be about as high as can be achieved by a simple procedure. Smaller ratios of citraconic anhydride to **1** have not been tried but could conceivably lead to very minor improvements in yield. A higher ratio (2.1) of citraconic anhydride to **1** simply led to a smaller yield of monocationic species and higher yields of di- and tricationic species in the SP-Sephadex column chromatogram. Other approaches to the synthesis of **2** are available and have been considered, but they do not offer any improvement in overall yield while requiring more labor. For example, we might choose to activate the free carboxyl group in mono(*N*-succinyl)-**1** (Reardon & Frey, 1984) to the *N*-hydroxysuccinimide ester and then use this to acylate a cluster with two free amino groups such as nondeca(*N*-acetyl)-**1**. As a matter of practicality it would be necessary to use a substantial molar excess of nondeca(*N*-acetyl)-**1** to minimize acylation of both amino groups in a given molecule. For this reason, and also because mono(*N*-succinyl)-**1** and nondeca(*N*-acetyl)-**1** are available in limited yields from **1**, the overall yield by such a procedure is likely to be smaller than the 13% achieved here. The purification also would not be simpler, since similar separation problems would exist. We note further that the present method also produces icosa(*N*-acetyl)-**1** in the second peak of Figure 2B in 11% yield as a useful side product.

The dimeric cluster **2** can be prepared as an alkylating reagent with a bromoacetyl or maleimide group by the same procedures described for the derivatization of the amino group associated with icosa(*N*-acetyl)-**1** (Yang et al., 1984). It could also be prepared as an acylating agent after carboxymethylation of the free amino group by reaction with bromoacetate or iodoacetate. The carboxyl group in the resulting *N*-(carboxymethyl)-**2** can be activated for use as an acylating agent, for example, as the *N*-hydroxysuccinimide ester, by using standard procedures (Reardon & Frey, 1984). The amino group in **2** can also be attached to biologically specific

ligands by reaction with alkylating or acylating derivatives of the ligands.

As pointed out in the introductory statement, dimeric undecagold clusters such as that described here may be useful in double-labeling scanning transmission electron microscopic experiments, in which one component of a complex structure is labeled with a monomeric cluster and another component with a dimeric cluster. In addition, as labeling reagents for use in low-dose conventional transmission electron microscopy, they should be superior to monomeric undecagold clusters.

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Synthesis and Biochemical Evaluation of 2′-Deoxy-*lin*-benzoadenosine Phosphates†

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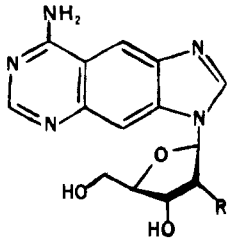
ABSTRACT: 2′-Deoxy-*lin*-benzoadenosine has been synthesized via reductive deoxygenation of 2-(β-D-ribofuranosyl)-8-(methylthio)imidazo[4,5-*g*]quinazoline. The 5′-mono-, 5′-di-, and 5′-triphosphates have been prepared by chemical and/or enzymatic methods. The 5′-diphosphate was found to be a substrate for phosphorylation by pyruvate kinase and was compared with various natural and extended substrates in kinetic assays. When 2′-deoxy-*lin*-benzoadenosine 5′-triphosphate was tested in a nick-translation experiment with *Escherichia coli* DNA polymerase I, a very low level of ^{32}P incorporation from [α-^{32}P]TTP into poly[d(AT)] was observed. Nearest-neighbor analysis indicated that the analogue was not significantly incorporated into internal positions in the polymer. In DNA-sequencing reactions, the analogue caused chain termination at adenine residues, although termination was less uniform and less efficient than that with 2′,3′-dideoxy-ATP. These experiments show that *lin*-benzoadenine can form a widened Watson–Crick base pair with thymine. They strongly suggest, though they do not prove, that the enzyme is able to attach the analogue to DNA.

The utility of dimensionally altered nucleoside and nucleotide analogues as specific biological probes of nucleotide–enzyme interactions has been demonstrated (Leonard, 1982). Specifically, the synthesis and biochemical evaluation of *lin*-benzoadenosine (**1**) and of several ribonucleotides and cofactors



1 R=OH lin-benzoadenosine

2 R=H 2′-deoxy-lin-benzoadenosine

containing this "stretched-out", fluorescent analogue of adenosine have provided useful information concerning the steric requirements for recognition by selected enzymes, the nature of the binding, and the relative position of hydrogen-bonding sites involved in recognition. As an extension of our previous work in this area, we have now prepared 2′-deoxy-*lin*-benzoadenosine (**2**) and its phosphate derivatives for evaluation as enzyme substrates or inhibitors. As potential substrates or inhibitors of enzymes that replicate or repair DNA, these compounds may provide insights into the steric requirements for deoxyribonucleotide recognition during DNA synthesis and into the mechanism of recognition of damaged DNA and its repair.

The synthesis of 2′-deoxyribonucleoside analogues traditionally has been a cumbersome task. Although phase-transfer catalysis of base deoxyribosidation with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranose and short reaction time favors β anomer formation (Winkeler & Seela, 1983), direct deoxyribosidation with a halo sugar derivative of 2-deoxyribose generally gives mixtures of α and β anomers, as well as positional isomers. This is attributed to the lack of anchimeric assistance that explains the stereospecificity of similar β-ribosidation reactions employing an acyl protecting group on the 2-hydroxyl of the corresponding ribose derivative (Watanabe et al., 1974). In order to avoid this problem by utilizing the readily available β-ribonucleosides, we have previously explored methods for their direct 2′-deoxygenation (Lessor & Leonard, 1981). Free-radical reduction of a thiocarbonyl derivative was selected as the best available method (Barton & McCombie, 1975; Barton & Subramanian, 1977), and a selective deacylation procedure was chosen to differentiate the three hydroxyl groups present in the original

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